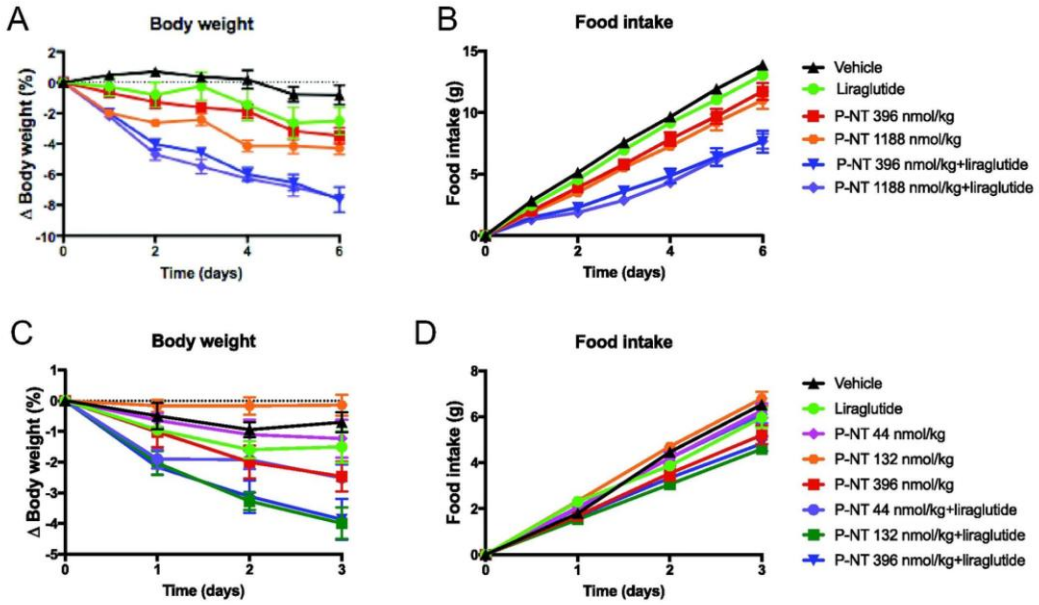
SUPPLEMENTARY DATA

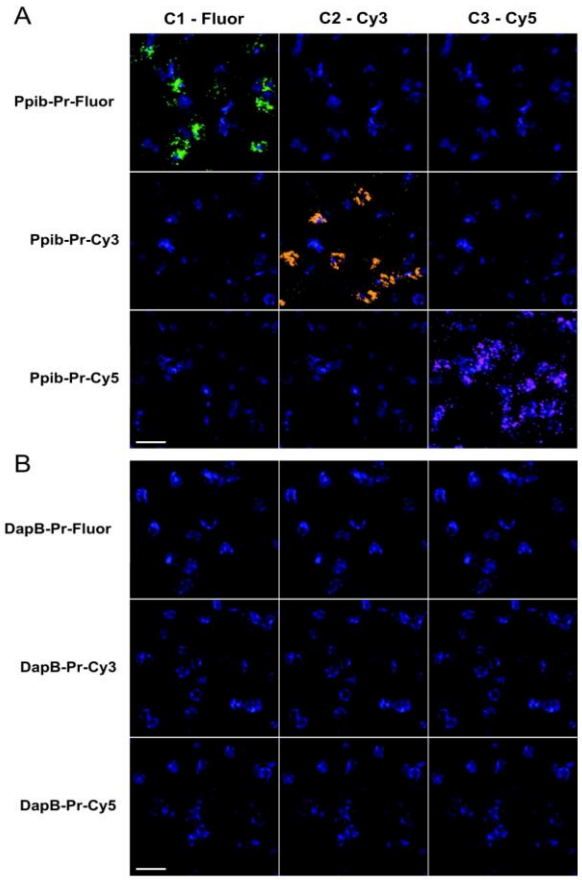
Supplementary Figure S1. Dose optimization of the P-NT dose A) Body weight and B) Food intake during 6 days of treatment for vehicle, liraglutide (8 nmol/kg), P-NT (396 nmol/kg), P-NT (1188 nmol/kg), P-NT (396 nmol/kg) + liraglutide (8 nmol/kg) and P-NT (1188 nmol/kg) + liraglutide (8 nmol/kg), C) Body weight and D) Food intake during 3 days of treatment for vehicle, Liraglutide (2 nmol/kg), P-NT (44 nmol/kg), P-NT (132 nmol/kg), P-NT (396 nmol/kg), P-NT (44 nmol/kg) + liraglutide (2 nmol/kg), P-NT (132 nmol/kg) + liraglutide (2 nmol/kg) and P-NT (396 nmol/kg) + liraglutide (2 nmol/kg). Data are mean ± SD. n=6.



SUPPLEMENTARY DATA

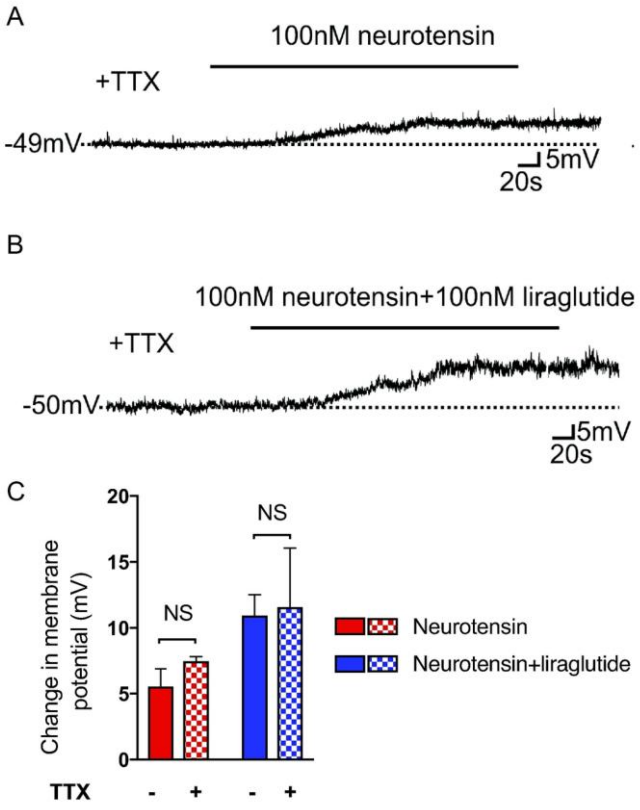
Supplementary Figure S2. Multiplex fluorescence *in situ* hybridization (M-FISH) control studies in the murine arcuate nucleus.

A) Representative confocal images of the arcuate nucleus section stained with probes specific for the housekeeping gene (positive control) *Mus musculus* Peptidylpropyl isomerase B (Ppib) mRNA with either fluorescein (Fluor), Cyanine3 (Cy3) or Cyanine5 (Cy5) demonstrating no or negligible crosstalk between the respective channels. B) Same as above, however stained with probes specific for *Bacillus subtilis* dihydrodipicolinate reductase (DapB) mRNA as a negative control demonstrating no staining in any channels. Nuclei was visualized with DAPI counterstaining (blue). Scale bars, 20 µm. n=3 mice.



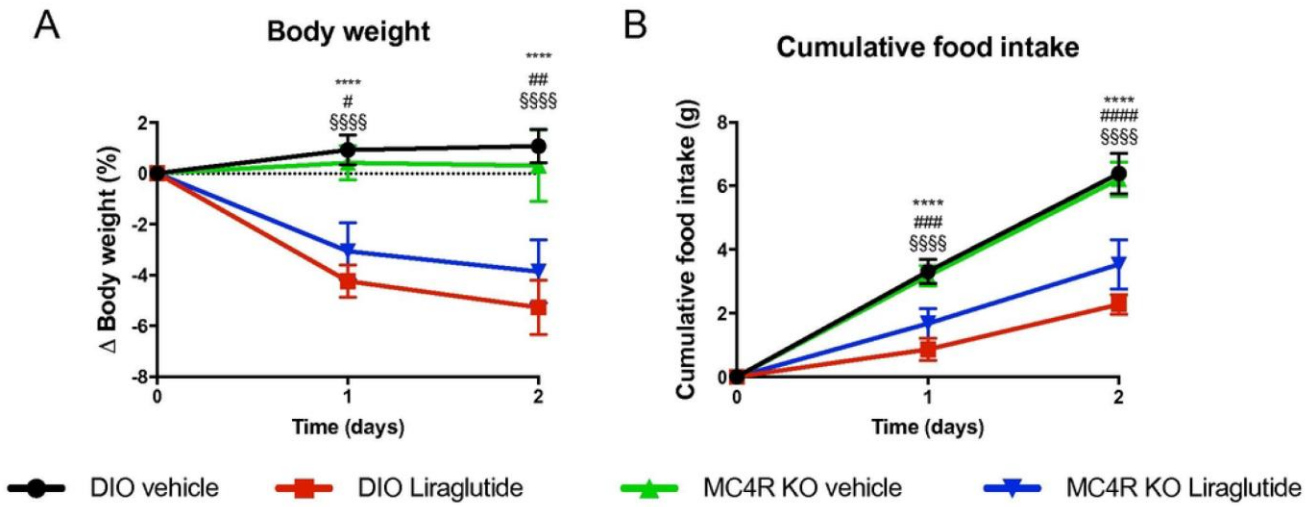
SUPPLEMENTARY DATA

Supplementary Figure S3. Depolarization of Pomc neurons by neurotensin and liraglutide in the presence of tetrodotoxin. A) Representative electrophysiological trace demonstrating that the NT induced depolarization of Pomc-hrGFP neurons persists in the presence of TTX, B) Representative electrophysiological trace demonstrating that the NT and liraglutide induced depolarization of Pomc-hrGFP neurons persists in the presence of TTX, C) NT and NT+liraglutide induced changes of membrane potential of POMC neurons with pretreatment of TTX. Error bars indicate SD. Data tested with Mann-Whitney non-parametric test n=4-5.



SUPPLEMENTARY DATA

Supplementary Figure S4. Effect of high dose liraglutide mono-therapy in MC4R KO mice compared to DIO controls. A) Body weight, B) Cumulative food intake. #p<0.05, ##p<0.01, ###p<0.001, ****/####/§§§§p<0.0001. **** represent differences between DIO vehicle and DIO liraglutide, #/##/###/#### represent differences between DIO liraglutide and MC4R KO liraglutide, §§§§ represent differences between MC4R KO vehicle and MC4R KO liraglutide. Data tested with two-way ANOVA repeated measurements with Tukey's multiple comparison test for individual time points. Data are mean ± SD. n=6.



SUPPLEMENTARY DATA

Supplementary Table S1. Primers

Gene	Forward primer	Reverse primer
HPRT	CTTTGCTGACCTGCTGGATT	TTTCCAGTTAAAGTTGAGAGATCA
TBP	TCAAACCCAGAATTGTTCTCC	GGTAGATGTTTTCAAATGCTTCA
LDLR	TCAGACGAACAAGGCTGTCC	CCATCTAGGCAATCTCGGTCTC
LRP1	AACCTTATGAATCCACGCGC	TTCTTGGGGCCATCATCAGT
PCSK9	CACCCTGGATGCTGGTATCT	GACCTCTTCCCTGGCTTCTT
LIPC	ATGTGGGGTTAGTGGACTGG	TTGTTCTTCCCGTCCATGGA
IDOL	AGGACTGTCTCAACCAGGTG	TGCCTTGTCTGCTCCTGTAA
SORT1	ATCCCAGGAGACAAATGCCA	AACCTTCCGCCACAGACATA
Cyp7b1	TCTGGGCCTCTCTAGCAAAC	GCACTTCTCGGATGATGCTG
Cyp8b1	CAGCGGACAAGAGTACCAGA	TGGATCTTCTTGCCCGACTT
Cyp27A11	CTTCATCGCACAAGGAGAGC	CCAAGGCAAGGTGGTAGAGA
Cyp3A11	CTCTCACTGGAAACCTGGGT	TCTGTGACAGCAAGGAGAGG
SQLE	TGTTGCGGATGGACTCTTCT	GAGAACTGGACTGGGGTTGA
APOE	GATCAGCTCGAGTGGCAAAG	TAGTGTCCTCCATCAGTGCC
ABCA1	AAAACCGCAGACATCCTTCAG	CATACCGAAACTCGTTCACCC